Feeding behavior of *Frontonia leucas* (Ehrenberg) (Protozoa, Ciliophora, Hymenostomatida) under different environmental conditions in a lotic system

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ABSTRACT. The objective of this study was to record and describe the morphological changes and the ingestion mechanisms of *Frontonia leucas* (Ehrenberg, 1833) according to the food type and to relate the food ingested with the different environmental conditions in a lotic system, namely São Pedro stream, located in the municipality of Juiz de Fora, Minas Gerais, Brazil. We sampled three points on a monthly basis from August 2002 to June 2003, each of which receiving different levels of untreated sewage. We prepared culture media for the ciliate specimens containing filtered water from each point and the types of food observed inside *F. leucas* (cyanobacteria, diatoms, desmids and testate amoebas). We observed the ingestion mechanisms of *F. leucas in vivo*, under a phase contrast optical microscope, using instantaneous sampling and sequence sampling as behavior observation methods, noting the following parameters: dissolved oxygen concentration, pH, conductivity and water temperature. We noted the *F. leucas* ciliates ingesting diatoms and desmids at collection point 1 and filamentous cyanobacteria, testate amoebas (*Arcella* and *Centropyxis*) and rotifers at points 2 and 3. The present work records for the first time the ingestion of testate amoebas of the genus *Centropyxis* by *F. leucas*. We noted five ingestion mechanisms by *F. leucas* while feeding on cyanobacteria and testate amoebas of the genus *Centropyxis*, three of these related to the ciliary action and two involving physical changes in the cytoplasm. For ingestion of diatoms, desmid (*Closterium*) and *Arcella*, the mechanisms involving ciliary action alone were sufficient for ingestion, since these preys are smaller than the ciliate under study. The autecological data registered for *F. leucas* were 1.98-8.01 mg l^{-1} O_2, pH 6.9-8.73, 58-390 µS/cm and 19.5-26.2°C, confirming its ample ecological valence.
KEY WORDS. Ciliate; polluted stream; predator; prey.

RESUMO. **Comportamento alimentar de *Frontonia leucas* (Ehrenberg) (Protozoa, Ciliophora, Hymenostomatida) sob diferentes condições ambientais em um sistema lótico.** O objetivo do presente trabalho foi registrar e descrever as alterações morfológicas e os mecanismos de ingestão em *Frontonia leucas* (Ehrenberg, 1833), conforme o tipo de alimento, e relacionar o alimento ingerido às diferentes condições ambientais de um sistema lótico, o córrego São Pedro, localizado no Município de Juiz de Fora, Minas Gerais, Brasil. Foram amostrados mensalmente, de agosto de 2002 a junho de 2003, três pontos que recebem diferentes níveis de lançamento de esgoto doméstico *in natura*. Foram preparados meios de cultura dos espécimes do ciliado em estudo, contendo água filtrada de cada ponto e os tipos de alimento observados no interior de *F. leucas* (cianobactérias, diatomáceas, desmídeas e tecamebas). Os mecanismos de ingestão de *F. leucas* foram observados *in vivo*, sob microscópio óptico de contraste de fase, utilizando-se como métodos de observação do comportamento a amostragem instantânea e a amostragem de seqüência. Registraram-se os seguintes parâmetros: teor de oxigênio dissolvido, pH, condutividade elétrica e temperatura da água. Os ciliados da espécie *F. leucas* foram observados ingerindo diatomáceas e desmídeas no ponto 1 de coleta e cianobactérias oscilatórias, tecamebas (*Arcella e Centropyxis*) e rotíferos nos pontos 2 e 3. O presente trabalho registra pela primeira vez a ingestão de tecamebas do gênero *Centropyxis* por *F. leucas*. Foram observados cinco mecanismos de ingestão realizados por *F. leucas* ao se alimentarem de cianobactérias e de tecamebas do gênero *Centropyxis*, sendo três relacionados com a ação ciliar e dois envolvendo mudanças físicas no citoplasma. Para a ingestão de diatomáceas, desmídeas e tecamebas do gênero *Arcella* somente os mecanismos que envolvem a ação ciliar foram suficientes para a ingestão, uma vez que estes alimentos são menores que o ciliado em estudo. Os dados de auto-ecologia registrados para *F. leucas* foram 1,98-8,01 mg l^{-1} O_2, pH 6,9-8,73, 58-390 µS/cm e 19,5-26,2°C, confirmando sua ampla valência ecológica.
PALAVRAS-CHAVE. Ciliado; córrego poluído; predador; presa.

Frontonia leucas (Ehrenberg, 1833) is found in running and stagnant water throughout the year, living in sediments at depths of up to five centimeters, and also in plankton. Although it forms resting cysts, there are no reports of its occurrence in terrestrial habitats. It is rare in marshlands, estuaries and sewage treatment plants. It is a widely distributed species (FOISSNER *et al*. 1999). According to BEERS (1933) and FOISSNER *et al*. (1999), it feeds on bacteria and heterotrophic and autotrophic flagellates, diatoms, algae up to 50 µm across, naked amoebas (*Amoeba proteus* Leidy 1878), testate amoebas (*Arcella, Difflugia*), ciliates (*Coleps, Aspidisca*) and even small metazoans (rotifers). DEVI (1964) reported cannibalism in this species.

The most important characteristic in identifying *F. leucas* is its single contracting vacuole with long collecting canals and the small oral apparatus when compared to its body size (FOISSNER *et al*. 1994, 1999). GOLDSMITH (1922) observed alterations in its original oval body shape according to the food ingested and the various mechanisms involved in ingesting desmids and cyanobacteria. BEERS (1933) described the ingestion mechanisms of one amoeba, *A. proteus*.

Frontonia leucas is included in the saprobic system, indicating environments that range from betamesosaprobic to alphamesosprobic. Because it is a eurythermic, euryoxibiontic and omnivorous species, it can be found in diverse environments and tolerates wide variations of physical and chemical parameters (FOISSNER *et al*. 1999).

The objective of this study was to record and describe the morphological changes and the ingestion mechanisms of *F. leucas,* according to the type of food, and to relate the food ingested with the different environmental conditions in a lotic system, São Pedro stream, located in the municipality of Juiz de Fora, Minas Gerais, Brazil.

MATERIAL AND METHODS

In the present study we collected monthly samples from August 2002 to June 2003 from three points along São Pedro stream in the municipality of Juiz de Fora, Minas Gerais, Brazil, in order to obtain samples with different levels of raw domestic sewage. Point 1 (UTM = 663036, 7590303) receives a low sewage inflow, while points 2 (UTM = 668307, 7591772) and 3 (UTM = 668645, 7592804) receive high sewage loads. We obtained sediment samples using 300-mL beakers. We then took the samples to the laboratory, placed them in Petri dishes and examined them under a stereoscopic microscope. We selected the specimens of *F. leucas* using micropipettes and observed them under an optical microscope (Olympus BX 41), taking digital photomicrographs of the specimens that showed some type of food in the cytoplasm. We then prepared cultural media of the specimens containing filtered water from each collection point and the types of food observed inside them (cyanobacteria, diatoms, desmids and testate amoebas). To observe the morphological changes and the ingestion mechanisms *in vivo*, we selected specimens of *F. leucas* and the foods from the culture media and transferred them to wet slides for observing living ciliates (TUFFRAU 1959) and observed them under a phase contrast optical microscope. The observation techniques employed were instantaneous sampling and sequence sampling, according to ALTMANN (1974), as adapted for the study of protozoans by D'AGOSTO *et al*. (2003).

We monitored the physical and chemical qualities of the water at each collection point with portable equipment, recording the dissolved oxygen concentration, pH, conductivity and water temperature. Statistical treatment of the physical and chemical data was by multivariate distance analysis (Euclidean distance).

RESULTS AND DISCUSSION

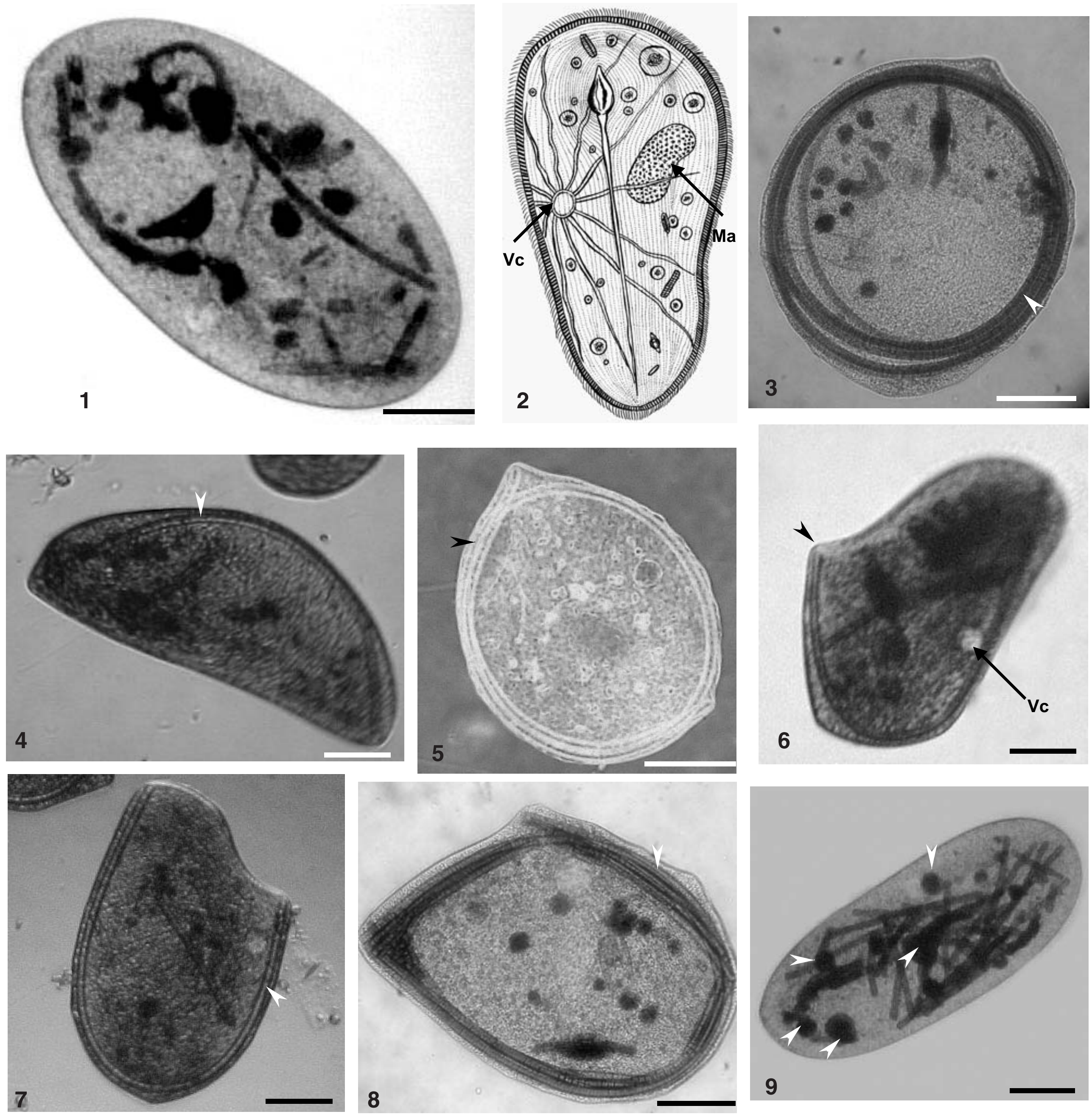
We observed the ciliates of *F. leucas* (Figs 1-2) ingesting filamentous cyanobacteria (Figs 3-9), testate amoebas (*Arcella* Ehrenberg, 1832 and *Centropyxis* Stein, 1857) (Figs 10-11) and rotifers (Fig. 12) at points 2 and 3 and diatoms (Fig. 13) and desmids (*Closterium* Nitzsch) (Fig. 14) at collection point 1. This work presents the first report of the ingestion of testate amoebas of the genus *Centropyxis* by *F. leucas* (Fig. 11), and the first record by photomicrographs of the morphological changes in *F. leucas* caused by the food ingested.

Morphological changes

The various types of food ingested by *F. leucas* cause alterations in their size and body shape (GOLDSMITH 1922). In the present study, there were morphological changes when the ciliates were feeding on cyanobacteria and testate amoebas of the genus *Centropyxis*. When the specimens fed on cyanobacteria (blue-green algae), we observed the following forms: circular (Fig. 3-5), semi-circular (Fig. 4), U-shaped (Fig. 7) and asymmetric (Fig. 8). GOLDSMITH (1922) described some similar shapes after *F. leucas* ingested cyanobacteria of the species *Oscillatoria prolifica* Gomont, 1892. In the present study, we noted that the ingestion of testate amoebas of the genus *Centropyxis* caused a slight widening of the body (Fig. 11), as recorded by BEERS (1933) while observing the ingestion of *Amoeba proteus* by *F. leucas*. The ingestion of diatoms and desmids did not cause any morphological alterations in *F. leucas*.

Ingestion mechanisms

All five of the ingestion mechanisms of *F. leucas* when feeding on cyanobacteria and algae described by GOLDSMITH (1922) were observed in the present work. Of these, three were related to the ciliary action: (1) pull exerted on the incoming food by the circumoral cilia, (2) movement of the ciliate in the direction of the food through the action of its own body cilia, and (3) a series of orderly changes in the position of the ciliate with reference to the food which permits the food to pass posteriorly with greater facility along the aboral surface of the ciliate. The other mechanisms involved physical changes in the cytoplasm: (4) superficial contractions of the aboral surface of the ciliate, forming tension points, and (5) cyclosis.



Figures 1-9. Morphological changes and ingestion mechanisms in *Frontonia leucas*: (1) the original oval body shape; (2) schematic drawing *in vivo*; (3 and 5) circular form; (4) semi-circular form; (7) U-shaped form; (6 and 8) asymmetric form;(3-5, 7-8) the arrows marks the cyanobacteria ingested; (6) formation of tension points (arrow); (9) spherical food vacuoles (arrows) containing an irregular mass. (Vc) Contracting vacuole, (Ma) macronuclear. Bars = 50 µm.

We observed the five mechanisms of ingesting cyanobacteria already described by Goldsmith (1922) and Beers (1933). We noted the formation of tension points when the specimens were ingesting cyanobacteria (Fig. 6). Although digestion is separate from the ingestion mechanisms, we observed the breaking-up of filamentous cyanobacteria and the formation of small spherical food vacuoles (Fig. 9) containing an irregular mass. Goldsmith (1922) mentioned this digestion step, but did not

Figures 10-14. *Frontonia leucas* in ingesting: (10) a testate amoeba *Arcella* sp.; (11) a testate amoeba, *Centropyxis* sp.; (12) a rotifer; (13) a large desmid, *Closterium* sp.; (14) diatoms. Bars = 50 µm.

present an illustration. According to this author, the entire digestive process takes approximately six hours. In the study of the feeding behaviour of another raptorial ciliate that feeds on cyanobacteria, *Pseudomicrosthorax dubis* (Maupas, 1883) Penard, 1922, was reported that the mechanisms for recognizing and ingesting cyanobacteria of the genus Oscillatoria are related to the influx of Ca^{+2} and efflux of K^{+} (RADEK & HAUSMANN 1996).

We verified that the specimens in the present study, while ingesting testate amoebas, employed the same five mechanisms described by GOLDSMITH (1922) and confirmed by BEERS (1933) in observing the ingestion of *A. proteus* by *F. leucas*.

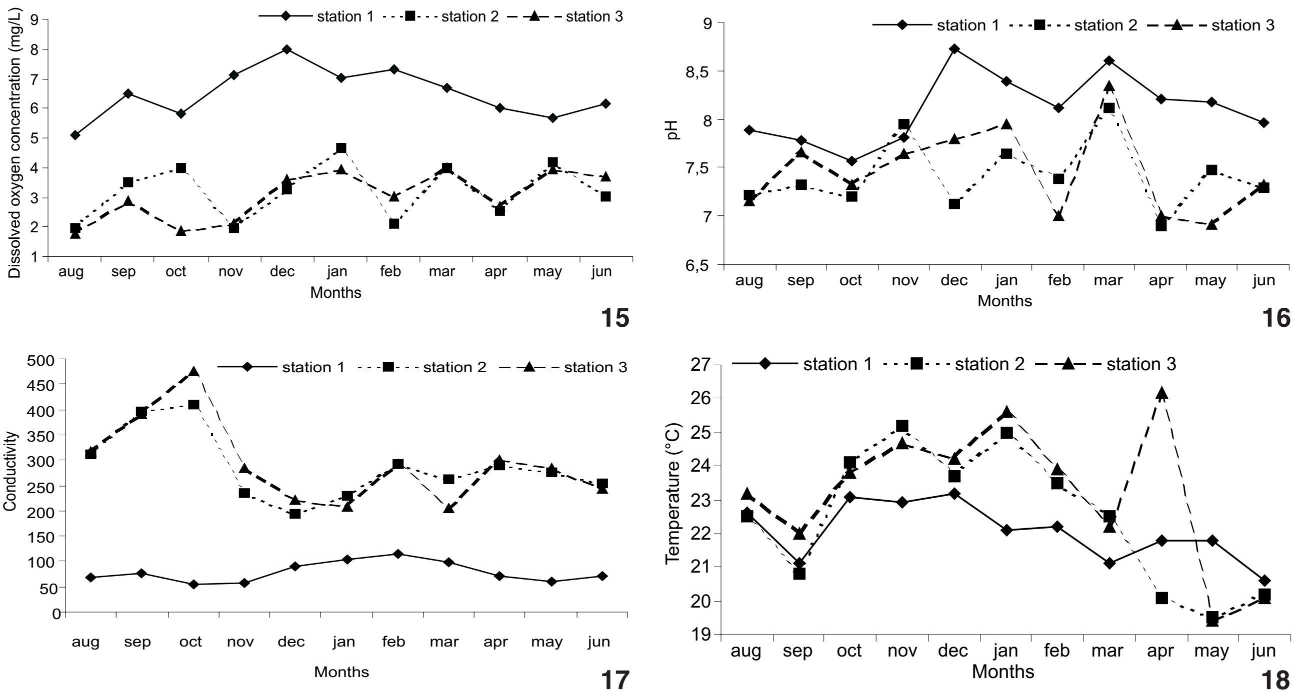
For ingestion of diatoms and *Arcella,* the three mechanisms involving ciliar action were sufficient, since these foods are smaller than *F. leucas*. GOLDSMITH (1922) reported that when the prey ingested is smaller than the ciliate, mechanisms involving physical changes in the cytoplasm are not necessary.

Feeding habits and environmental conditions

We observed the ciliates of the species *F. leucas* ingesting diatoms and desmids at collection point 1 and filamentous cyanobacteria, testate amoebas (*Arcella*, *Centropyxis*) and rotifers at points 2 and 3. The availability of food is an important biotic factor that controls the distribution of protozoan populations in various ecosystems (NOLAND 1925, PRIMC 1988, PRIMC-HABDIJA *et al*. 1998, MADONI & BASSANINI 1999), but the omnivorous dietary habit of *F. leucas* allows it to live both in the headwaters and polluted stretches of various lotic systems, as registered in the present work and by other authors (CZAPIK 1982, SPARAGANO & GROLIÈRE 1991, PACKROFF & ZWICK 1996, SOLA *et al.* 1996, MADONI & BASSANINI 1999). In São Pedro stream, we noted *F. leucas* at points with different raw sewage levels, in agreement with the findings of CZAPIK (1982), SPARAGANO & GROLIÈRE (1991) and MADONI & BASSANINI (1999).

Another factor that influences the ciliate community is the level of sensitivity of the various species of these organisms to the physical and chemical properties of the water. This factor can be explained by the specific demands of various ciliate protozoa in relation to water characteristics such as amount of dissolved organic matter, temperature, pH, conductivity and dissolved oxygen concentration (NOLAND 1925, KUDO 1967, SLEIGH 1988). The three sample points were different in relation to the physical and chemical characteristics (Figs 15-18), with points 2 and 3 being most similar (Fig. 19). These results confirm the high sewage loads at points 2 and 3 due to dense human occupation in the region (Fig. 19). The minimum and maximum values of the physical and chemical parameters recorded in the samples of *F. leucas* collected were: 1.98-8.01 mg l^{-1} O_2, pH 6.9-8.73, 58-390 µS/cm and 19.5-26.2ºC, corroborating the autecological data presented by FOISNNER *et al*. (1999) for *F. leucas* in a survey article. The occurrence of *F. leucas* in environments with a wide range of physical and chemical parameters demonstrated that this species has low sensitivity to environmental variations.

Of the eleven collections at each sample point, we recorded *F. leucas* in eight samples from point 1 and five from points 2 and 3. MADONI & BASSANINI (1999), taking monthly samples during one year, studied six points of the Parma River in Italy, three of them located inside a natural park, two receiving low concentrations of sewage and one high concentrations. They recorded *F. leucas* seven times in the set of unpolluted and low-polluted points and not at all at the point with the



Figures 15-18. Variation of the physical and chemical qualities from three points along São Pedro stream, from August 2002 to June 2003: (15) dissolved oxygen concentration, (16) pH, (17) conductivity (µS/cm), and (18) temperature from three points along São Pedro stream, from August 2002 to June 2003.

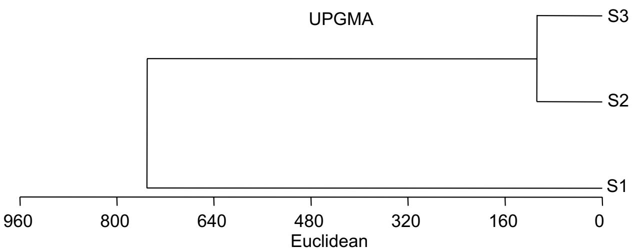


Figure 19. Dendrogram of cluster analysis, showing the similarities among stations on the basis of physycal and chemical qualities.

highest saprobic level, indicating this species' preference for alpha-beta mesosaprobic environments. The needs of these species and communities are complex and the disturbances caused by pollution can considerably alter the aquatic food chain (Madoni & Bassanini 1999). In oligosaprobic waters, the ciliates feed mainly on diatoms, while few feed on bacteria and cyanobacteria. As the saprobic level increases, the trophic structure is altered, causing a decrease in algivores and an increase in the number of bacterivore species. Omnivorous ciliates, such as *F. leucas*, become facultative bacterivores due to the abundance of bacteria in water with high sewage levels (Primc 1988). Because it is a species with an ample ecological valence and thus rapid ecological adaptability and wide tolerance for environmental changes, *F. leucas* has been registered at locations with different saprobic conditions.

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